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Molecular and Cytogenetic Analysis of the Fragile X Syndrome in a Series of 453 Mentally Retarded Subjects: A Study of 87 Families

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We report on a series of 453 mentally retarded subjects investigated for fragile X syndrome from 1982 to July 1995. The 22% rate of efficiency of FRAX positivity indicated a significant preselection by the clinicians. However, this rate dropped to 11% in the last year. Since 1992, Southern blot analysis was extended to include family members of the 87 positive subjects, for a total of 442 individuals examined with the probe StB12.3. In addition to premutated (118), fully mutated (148), and pre/full mutation mosaic subjects (27), 14 atypical cases were found. Some of these cases are described in more detail. In particular, we report on the hybridization and polymerase chain reaction data of 2 fragile X subjects with full mutation and a 2.8-kb allele and 1 with full mutation and a 2.4-kb allele. An intellectually normal male with 18% of fraXq27.3 and an unmethylated full mutation is also described. Finally, a mentally retarded child with only a lower allele of 2.7 kb is presented.

KEY WORDS: fragile X, DNA diagnosis, mosaics, atypical cases

INTRODUCTION

Fragile X syndrome is one of the most frequent genetic causes of mental retardation; the mutational event underlying the syndrome is an expansion of a trinucleotide repeat, p(CGG), at the FMR1 locus in the 5′ region of the gene [Kremer et al., 1991; Oberlé et al., 1991; Verkerk et al., 1991]. This expansion may be expressed as a fragile site at the distal long arm of the mutated X chromosome (Xq27.3). Since 1982, we have been performing fragile X diagnosis by cytogenetic analysis, linkage analysis, and, after the cloning of FMR1 gene, by the direct analysis of the expanded repeat.

From 1982 to July 1995, we examined 453 (354 male, 99 female) mentally retarded individuals for fragile X by using different available techniques. The investigation was extended to the families of the positive subjects.

MATERIALS AND METHODS

The subjects referred to our laboratory were individuals with mental retardation (MR), developmental delay, or behavioral problems; most of the people examined came from different regions in Italy, and their blood samples were sent to our laboratory by mail. In addition, several pregnant women with 1 or more undiagnosed MR subjects in their family were examined.

Cytogenetic Analysis

To exclude any chromosomal rearrangement, constitutional chromosome analysis was performed in all the propositi according to standard techniques; in addition, the following methods of fragile X induction were used: (1) TC199 + 5% AB serum and (2) TC199 + trimethoprim 15 μg/ml.

At least 100 metaphases were scored for male samples and 150 for female samples.

DNA Analysis

Genomic DNA was extracted by the phenol-chloroform method from 20 ml of EDTA (as anticoagulant) collected peripheral blood. Seven micrograms of

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DNA were EcoRI/EagI double digested; blotting and hybridization with the probe StB12.3 were carried out according to Rousseau et al. [1991].

A nonradioactive polymerase chain reaction (PCR) analysis of the FRAXA CGG repeat was performed in particular cases, as described by Wang et al. [1995], by using the primers FXC (5′ GCT CAG CTC CGT TTC GGT TTC ACT TCC GGT 3′) and FXF (5′ AGC CCC GCA CTT CCA CCA CCA GCT CCT CCA 3′). Twenty-five microliters of PCR product were analyzed by electrophoresis in a 3% agarose methaphore (FMC) containing ethidium bromide.

RESULTS

Our data on fragile X syndrome, collected over 13 years, are reported in Tables I and II. During this period, we used the different technical approaches available for the diagnosis of Martin–Bell syndrome (MBS): cytogenetic induction of FraXq27.3 site, linkage analysis, and direct analysis of the CGG repeat at FMR-1.

From 1982 to July 1995, 453 (354 male, 99 female) MR patients were examined (Table I). One hundred subjects (22%) were found to be positive for fragile X syndrome, and, in 87 cases, the analysis was extended to family members, for a total of 442 individuals examined with the probe StB12.3.

In addition, 19 pregnant women, with an undiagnosed and unavailable MR subject in their families, were analyzed with the probe StB12.3; 1 woman was found to be a carrier of a premutation and underwent prenatal diagnosis.

Eighty-seven families were analyzed in our laboratory (Table II). Among the 142 females at risk, we identified 58 carriers (26 premutation, 29 full mutation, 1 mosaic pre/full, and 2 atypical patterns). Among the 63 males at risk, 17% (11/63) were normal transmitting males, and 1 had an atypical hybridization pattern with an unmethylated full mutation.

All 97 obligate carriers were confirmed heterozygotes for a premutation (83%), a full mutation (11%), or mosaics/atypical patterns (5%).

Twenty-one carrier females (at-risk and obligate carriers) were pregnant and underwent prenatal diagnosis.

Of the mutated cases, 8.8% were pre/full mutation mosaics and 4.6% were atypical cases, including "methylation mosaics" (incompletely methylated full mutation; see Fig. 2), male mosaics with normal allele (or lower allele) + full mutation, Δ borderline (600 bp) with 100% mutated inactive X in female, and large unmethylated full mutation (Table III).

Figure 1 shows the hybridization patterns of 2 male mosaics with a normal allele: in these cases, we can exclude the possibility that the 2.8-kb band is due to DNA contamination because of the strong autoradiographic signal after an overnight exposure of the blots.

The hybridization pattern of 1 cases of male mosaics with a smaller allele (2.4 kb) is shown in Fig. 2; PCR analysis demonstrated an amplification product of 120 bp (Fig. 2); in the second case (data not shown), the absence of amplification might be due to a rearrangement involving the primer annealing sequences. Such findings may be the expression of the high instability of the CGG repeats that might undergo different rearrangements that involve adjacent sequences and lead to deletions.

TABLE I. Summary of Molecular and Cytogenetic Results

Years	Diagnostic approach[a]	Total propositi	Sex ratio: M:F	Positive cases: M:F	Total positive	
					No.	%
1982–1990	CKF	53	50:3	12:2	14	26
1991	CKF + LA	17	15:2	6:1	7	41
1992	CKF + MA	40	31:9	9:1	10	25
1993	CKF + MA	81	69:12	21:3	24	29
1994	CK + MA	155	116:39	32:2	34	22
July 1995	CK + MA	107	73:34	10:1	11	10
Total		453	354:99	90:10	100	22

[a] CK = constitutional karyotype; LA = linkage analysis; CKF = constitutional karyotype + FraXq27.3 induction; MA = mutation analysis (StB12.3).

TABLE II. DNA Diagnosis in 442 Subjects Belonging to 87 Families

	Normal	Premutation	Full mutation	Mosaics	Atypical	Total
Affected males			96	20	8	124
Affected females			12	3	1	16
Males at risk	51	11			1	63
Obligate carriers		81	11	3	2	97
Female at risk	84	26	29	1	2	142
Total	135	118	148	27 (8.8%)	14 (4.6%)	442

TABLE III. Numbers of Atypical Hybridization Patterns Reported

"Methylation mosaics"	7
Male mosaic with normal allele + full mutation	1
Male mosaic with normal allele + premutation + full mutation	1
Male mosaic with lower allele + full mutation	2
Large unmethylated full mutation	1
Borderline (Δ600 bp) with 100% of mutated inactive X in females	2
Total	14

Figure 3 shows the StB12.3 hybridization pattern of an intellectually normal man at risk. This shows an 18% cytogenetic expression of fraXq27.3. The DNA, tested first by PCR, did not demonstrate any amplification product; Southern blot analysis revealed a smear in the range of 3.1–4.6 kb (Δ = 300–1,800 bp of the CGG repeat), demonstrating a premutation/unmethylated full mutation mosaic. This result agrees with that of Rousseau et al. [1994], where the abnormal phenotype is more strongly associated with the presence of abnormal EagI methylation than with the exact size of the expansion at this locus.

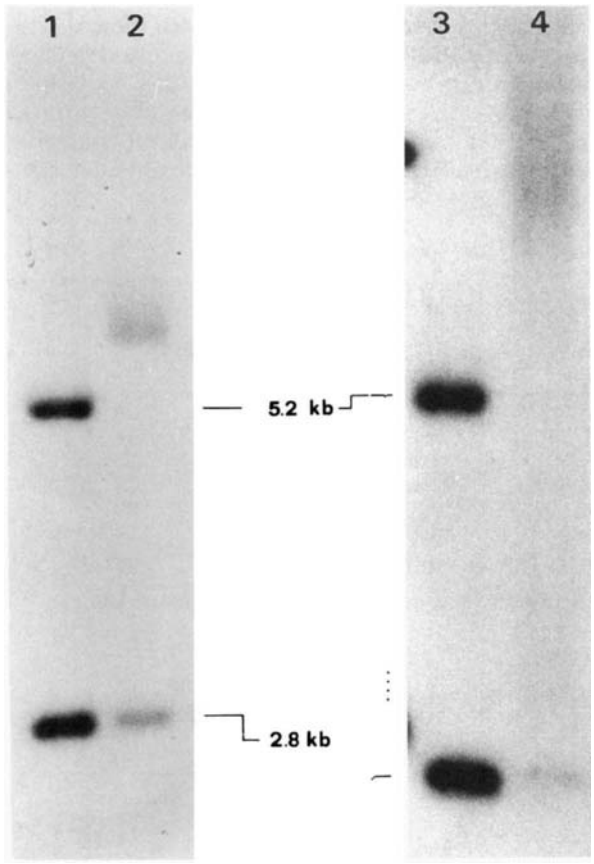

Fig. 1. **Lanes 1–4:** StB12.3 hybridization pattern of EcoRI/EagI double-digested DNA samples derived from 2 male mosaics with a normal allele (lanes 2 and 4).

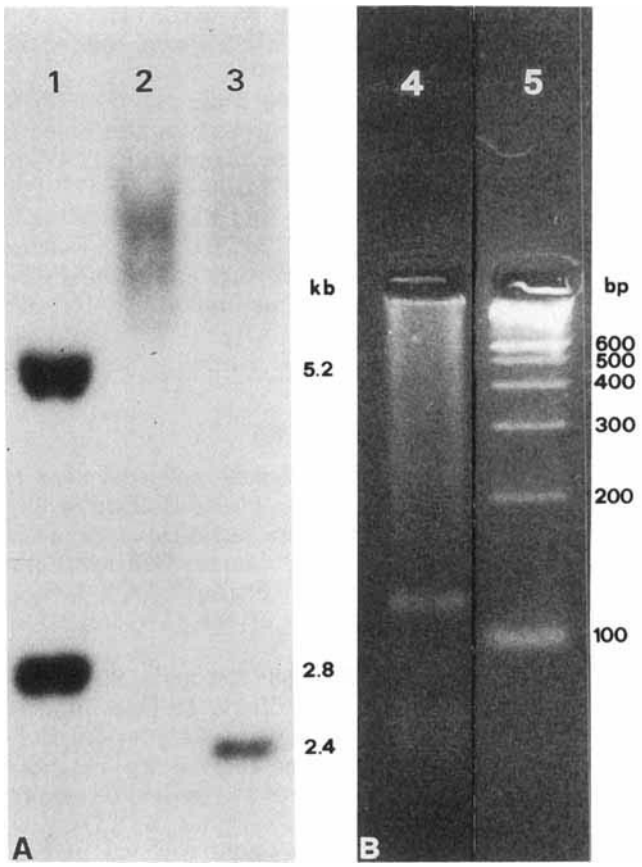

Fig. 2. StB12.3 hybridization pattern (**A**) and PCR analysis (**B**). **Lane 1:** Normal female control. **Lane 2:** Fragile X male showing an incompletely methylated full mutation. **Lane 3:** Fragile X male showing mosaicism with a 2.4-kb allele. **Lane 4:** The same sample as in lane 3, showing a 120-bp amplification product.

We examined a 2-year-old child who was referred to us because his clinical features were suggestive of fragile X syndrome; a 2.7-kb band was seen after EcoRI/EagI double digestion and StB12.3 hybridization (Fig. 4); no evidence of faint smear was demonstrated, even after several days of blot exposure, and no amplification products were seen in PCR analysis. We plan to test the sample by FMR-1 cDNA to verify the presence of a deletion and monoclonal antibodies [Willemsen et al., 1995] to verify the presence of FMR protein.

CONCLUSIONS

In recent years, and in particular after the cloning of the FMR-1 gene, the fragile X syndrome has become a better known clinical entity. As a consequence, many more MR subjects, even without the clinical features of the syndrome, are sent for fragile X diagnosis.

The 22% mean incidence rate of fragile X in the mentally retarded is a striking result when compared with other studies [Kaplan et al., 1994; Rousseau et al.,

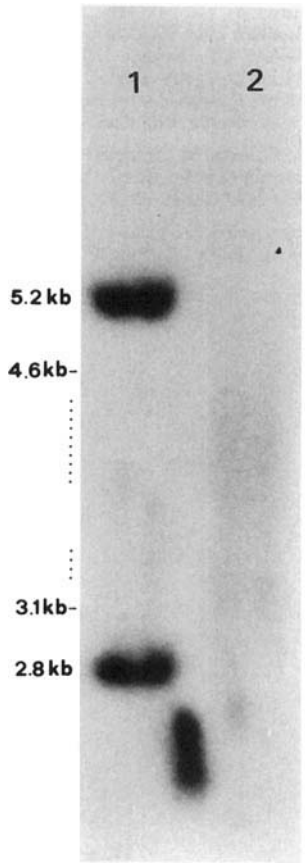

Fig. 3. StB12.3 hybridization pattern of EcoRI/EagI double-digested DNA. **Lane 1:** Normal female control. **Lane 2:** Premutation/unmethylated fully expanded mutation mosaicism of an intellectually normal man.

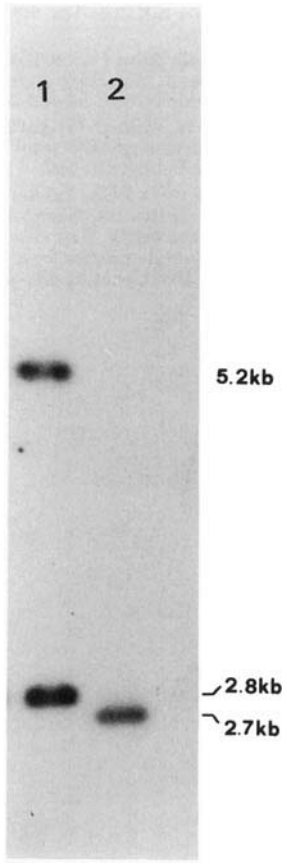

Fig. 4. StB12.3 hybridization pattern of EcoRI/EagI double-digested DNA. **Lane 1:** Normal female control. **Lane 2:** Mentally retarded child with a 2.7-kb allele.

1994; Ryynänen et al., 1994; van den Ouweland et al., 1994; Moutou et al., 1995; Turner et al., 1996]. This finding might be explained by a consistent preselection at the clinical level, as most of the patients were referred to us by geneticists. However, in the last year, the efficiency rate dropped to 11%.

To establish the precise frequency of fragile X syndrome, it will be necessary to perform a screening program in MR and normal subjects.

The finding of a relevant number of atypical cases highlights the problem that the correct diagnosis of fragile X syndrome is not always possible by a single method. Incorrect genetic counseling may be the consequence of a wrong diagnosis.

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